Comparative Biochemistry and Physiology, Part A 252 (2021) 110824

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Graphical review

The biological legacy of sulfur: A roadmap to the future

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ARTICLE INFO

Keywords:
Reactive oxygen species
ROS
Reactive sulfur species
RSS
Oxidative stress
Evolution
Sulfur metabolism
Antioxidants

ABSTRACT

"Nothing in biology makes sense except in the light of evolution" (*Theodosius Dobzhansky*) and "For such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied" (*August Krogh*); dictums that can be used to illustrate the past and provide a guide to the future. Although sulfur was integral in the origin of life, and nearly seven-eights of subsequent evolution, its physiological importance is largely overlooked because much of contemporary life it is based on oxygen and the adherent problems associated with oxygen deficit (hypoxia) or excess (oxidative stress). This graphical review will summarize sulfur's role in evolution and make a case that many of the regulatory activities attributed to oxygen and reactive oxygen species (ROS) can also be ascribed to reactive sulfur species (RSS). ROS and RSS are chemically similar and signal via identical cysteine residues on regulatory proteins and have identical downstream effector responses. Antioxidant mechanisms, generally attributed to the advent of an oxic existence, actually appeared over 2 billion years prior, in sulfur metabolizing organisms. Recent evidence suggests they are active in sulfur metabolism to this day. Understanding these aspects of ROS and RSS suggests that alternative mechanisms for oxidant/antioxidant pathways and therapies must be considered. As oxygen and reduced sulfur do not coexist, either in cells or the environment, it is also important to design and conduct experiments in oxygen levels that are physiologically relevant. For every experiment there are optimal conditions under which it must be studied.

1. Introduction

Oxygen and sulfur are chemically similar and recent evidence suggests that reactive oxygen species (ROS) and reactive sulfur species (RSS) have similar, or nearly identical signaling attributes (Olson 2020). However, ROS and RSS do not coexist chemically and it has been difficult to discern their relative roles in cell function. This brief review will examine the biological origins of ROS and RSS, their chemistry and signaling properties, and show how confusion can arise in distinguishing between the two, especially in the supraphysiological oxygen environments in which most experiments are conducted.

2. Life's beginnings

Life began in an anoxic world. Organic molecules could have been, and likely were, produced by lightning strikes in a 'primordial soup' (Miller 1953), delivered from extraterrestrial sources or the atmosphere (Pizzarello and Shock, 2010, Sagan and Khare 1971) or generated within the earth in the caldrons of deep-sea hydrothermal vents (Cody 2004; Wächtershäuser 1988). But of these options only the latter could sustain life by providing a dependable source of energy. Reducing equivalents in the form of hydrogen sulfide (H_2S) were continuously delivered to a more oxidizing ocean floor - where they created a dependable redox gradient around which life evolved and depended (Fig. 1). While these early chemoautotrophs used the reducing power of H_2S to reduce inorganic molecules such as CO_2 to methane, it wasn't long until other organisms developed metabolic pathways to reverse these reactions and a biological redox sulfur cycle was established. Once light-gathering antennae were developed to use photons, presumably to oxidize H_2S and reduce carbon (Olson 2019), life no longer depended on the redox gradient around the vents and was free to spread and expand. It was now necessary to establish some sort of redox balance between sulfur oxidizing and sulfur reducing organisms to ensure the survival of both. It is not yet completely known how this was accomplished but the appearance during this time of sulfur buffers (e.g. glutathione) and sulfur- and selenium-based electron shuttles such as thioredoxin/thioredoxin reductase (Trx/TrxR) glutathione/glutathione reductase (Grx/GrxR) and peroxiredoxins (Prx) suggest a solution to the problem. Work

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https://doi.org/10.1016/j.cbpa.2020.110824
Received 20 September 2020; Received in revised form 17 October 2020; Accepted 22 October 2020
Available online 30 October 2020

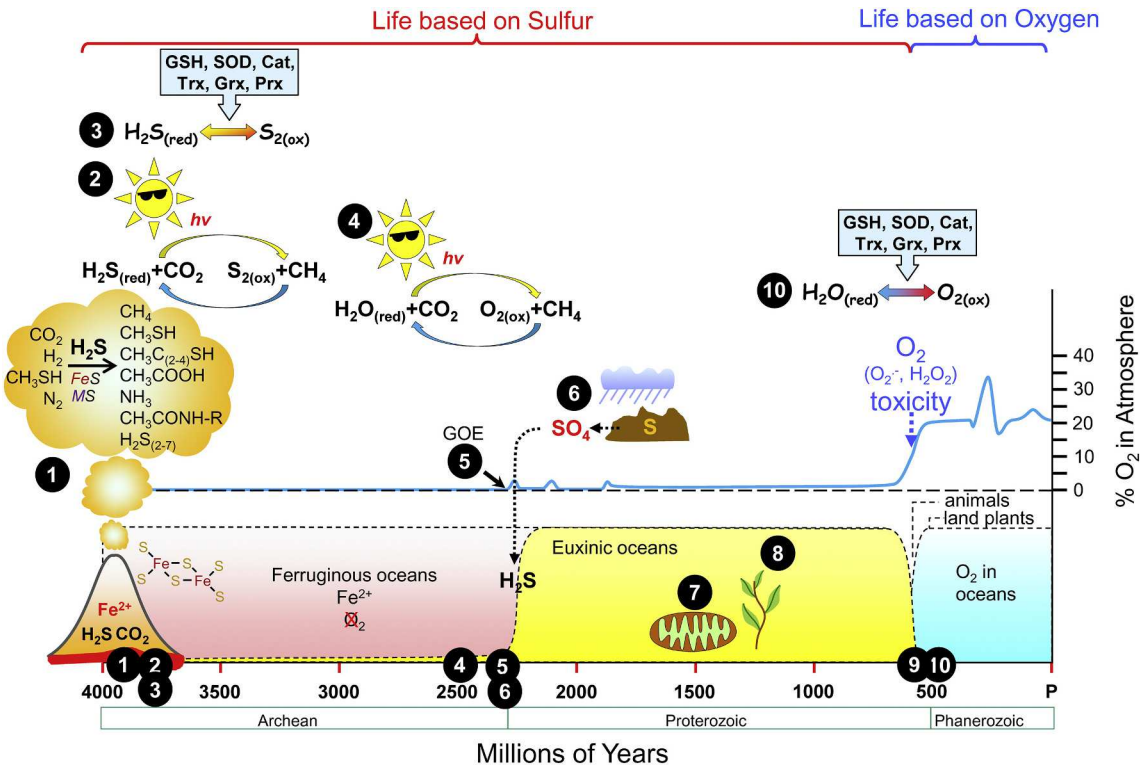

Fig. 1. The biological legacy of sulfur. (1) Life originated approximately 3.8 billion years ago (bya) in undersea hydrothermal vents that sat atop massive sulfur deposits and liberated hydrogen sulfide (H_2S), carbon dioxide (CO_2) and reduced transition metals. Intense pressure and heat from underlying magma and reducing equivalents from H_2S drove a variety of organic reactions whose products became stable as the plume rose, cooled and entered the anoxic ferruginous (Fe^{2+}-rich) ocean. H_2S reactions with reduced iron and other transition metals (FeS and MS) formed primordial catalysts and mineral substrates that anchored reactions and created early prototypical membranes, ultimately becoming the iron sulfur clusters (Fe_2S_6) that conduct electrons in modern cells. These sulfur-oxidizing organisms were soon complemented by sulfur-reducing organisms and a sulfur-cycling biome appeared, energized by the reducing, electron-donating, H_2S from the vents. (2) Anoxygenic photosynthesis appeared within a hundred or so million years using solar energy to drive H_2S/CO_2 oxidation/reduction reactions and thereby freeing organisms from the tether of the vents, and the sulfur redox biome expanded. (3) Attendant with this chemistry was the necessity to control these redox reactions and complex sulfur redox regulators appeared including redox buffers (e.g, glutathione), sulfur-regulating enzymes superoxide dismutase (SOD) and catalase (Cat) and electron-conducting sulfur and seleneosulfur enzymes thioredoxin, glutaredoxin and peroxiredoxin (Trx, Grx, Prx) systems. (4) It took nearly a billion years for the light-gathering antennae to become sophisticated enough to capture sufficient energy to oxidize water to oxygen, a process that appeared in cyanobacteria and would change life forever. (5) Most oxygen produced by oxygenic photosynthesis was immediately reduced by ferrous iron in the oceans but a small amount escaped into the atmosphere leading to the 'great oxidation event' (GOE) around 2.3 bya that episodically increased atmospheric oxygen to 0.1–0.5%. (6) This oxidized terrestrial sulfur to sulfate that was washed to the sea and reduced to H_2S creating vast sulfidic and anoxic (euxinic) areas. (7) It was in this environment that an archeaon engulfed a α-protobacterium creating the first eukaryotes that would survive and develop in these euxinic waters for nearly a billion years more. (8) Plants appeared from endosymbiosis of cyanobacteria and (9) the cumulative effect of oxygenic photosynthesis by cyanobacteria and plants began to oxidize the oceans and, finally around 600 million years ago, the oceans became oxygenated and atmospheric oxygen rose toward present day levels. Survival was now supposedly dependent on creating oxygen de-toxifying mechanisms (10). However, these 'antioxidants' had already been present for over 3 billion years (3) and I propose that they were easily repurposed from sulfur to oxygen metabolism. Modified from Olson and Straub 2016, with permission. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in our lab (described below) showed that superoxide dismutase (SOD) and catalase (Cat) also metabolize sulfur, which is consistent with the appearance of these enzymes during this early period (Olson 2019).

It took another billion years for the light-gathering antennae in sulfur oxidizing organisms to become sufficiently sophisticated to capture enough energy to oxidize water to oxygen and concomitantly reduce carbon dioxide to organic carbon. Oxygen was essentially a byproduct and mostly consumed by the abundant ferrous iron (Fe^{2+}). As oceans became more sulfidic, a sulfur reducing archeon engulfed a sulfide oxidizing α-protobacteria and eukaryotes were formed, each cell now containing a redox sulfur cycle (Searcy 2003). These eukaryotes lived and evolved for the next billion years in an environment that was mostly anoxic and sulfidic. Along the way they incorporated a cyanobacterium, the first chloroplast. These new plants augmented the rate of oxygen production, eventually oxidizing oceanic H_2S and Fe^{2+}. Oceanic and atmospheric oxygen now began to rise toward present day levels.

3. Oxygen and the OxTox hypothesis

It is generally accepted that with the rise in oxygen and its potential toxicity, organisms were faced with three options, retreat to hypoxic/anoxic environments, develop mechanisms to detoxify oxygen, or die, the OxTox hypothesis (Kurland and Andersson 2000). I disagree with the first two of these scenarios, as described below, the third, is a common feature of evolution and not unique to oxygen toxicity.

First, organisms didn't retreat to hypoxic environments, they were already there, and abundantly so. They still are. In fact, it has been estimated that only 14% of all eukaryotes and only 0.001% of all microbes have been discovered to date (Locey and Lennon 2016; Mora et al. 2011). Why? Because we either don't or can't go there to find them as many of these inaccessible habitats are deep in the ocean or underground. They are also undoubtedly hypoxic or anoxic.

Second, I don't believe oxygen toxicity was as dire a threat as proclaimed. The OxTox hypothesis posits that survival was dependent on the development of oxygen detoxifying mechanisms, i.e., the

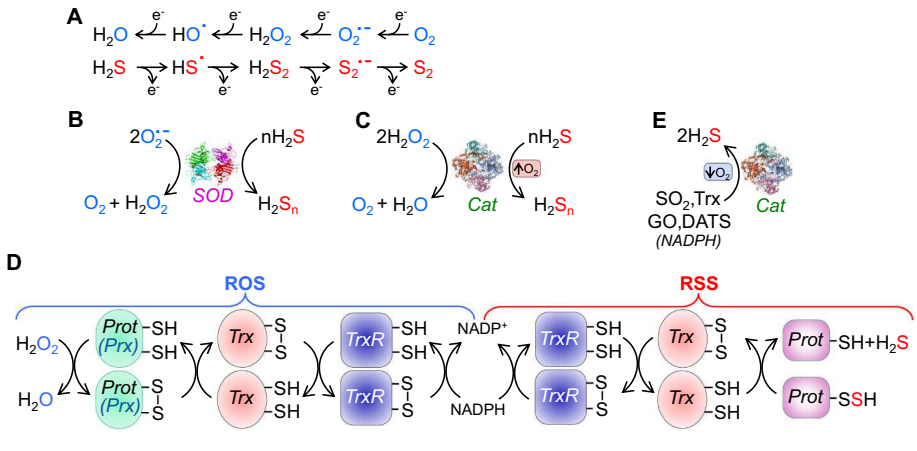

Fig. 2. Similarities between reactive oxygen species (ROS) and reactive sulfur species (RSS). (A) Chemical similarities: sequential one-electron reductions of O_2 produces superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and water. One electron oxidations of H_2S produces a thiyl radical ($HS^{\bullet}$), hydrogen persulfide (H_2S_2), persulfide radical 'supersulfide' ($S_2^{\bullet-}$) and elemental sulfur S_2; the latter often cyclizing to S_8. (B-D) Catabolic similarities: A number of 'antioxidant' enzymatic pathways inactivate ROS. (B) Superoxide dismutase (SOD) dismutes superoxide to peroxide and water, (C) catalase (Cat) dismutes peroxide to oxygen and water and (D) the thioredoxin/thioredoxin reductase (Trx/TrxR) electron shuttle transfers electrons from NADPH to reduce hydrogen peroxide or protein peroxides to water. SOD also catalyzes the oxidation of H_2S to *per*- and polysulfides (H_2S_n, n = 2–5) in the presence of oxygen or peroxide (B). In 21% oxygen Cat oxidizes H_2S to per- and polysufides (C), whereas in hypoxia Cat transfers electrons from NADPH and produces H_2S from a variety of sulfur bearing molecules including sulfur dioxide (SO_2), Trx, garlic oil (GO) and the garlic compound diallyl trisulfide (DATS; E). The Trx/TrxR system also reduces a protein persulfide to liberate H_2S (D).

'antioxidants' glutathione, SOD, Cat, Trx/TrxR, Grx/GrxR. Prx, and so on. But as discussed above, these were already well established to deal with sulfur nearly 3 billion years before oxygen was around. And, as will be discussed in the following sections, they needed only minor 'tweaking' to switch from sulfur to oxygen metabolism. So why was the rise in oxygen accompanied by an explosion in biomass? Firstly, oxygen is a better electron acceptor than H_2S is an electron donor and this oxygen-based metabolism greatly increases the efficacy of redox reactions. Secondly, and considerably more importantly, water is nearly everywhere. H_2S and organic sulfur were at best limited to geothermal sources, and with the appearance of oxygen, the range of sulfur-dependent organisms was even more restricted. With the advent of oxygenic photosynthesis, and an abundance of CO_2, life became as simple as 'just add water'. Plants oxidize water and reduce inorganic compounds, and animals reverse the process in obligate symbiosis. Without plants (and cyanobacteria) aerobic life will cease; a warning to us all.

4. Similarities between reactive oxygen species (ROS) and reactive sulfur species (RSS)

Oxygen toxicity is largely attributed to reduced oxygen species, superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($HO^{\bullet}$). These ROS are derived from sequential one-electron reductions of O_2, culminating in water. One-electron oxidation of H_2S produces similar molecules (in reverse) with sulfur substituting for oxygen (Fig. 2A).

There are numerous similarities between ROS and RSS. Many of these are probably due to the chemical similarities between sulfur and oxygen. Sulfur sits directly beneath oxygen on the periodic table and both chalcogens have six valence electrons. However, sulfur is a larger atom (Van der Waals radius of 1.80 vs 1.42 Å, respectively), it has lower electronegativity (2.58 vs 3.44, respectively on the Pauling scale) and it is more polarizable (Benchoam et al. 2019). Because sulfur's electrons are farther from the positive nucleus, sulfur's electrons tend to be more promiscuous lending to greater bioreactivity.

Recent studies have shown that metabolic 'antioxidant' systems act upon both ROS and RSS (Fig. 2B-D). These include both cytosolic and mitochondrial superoxide dismutases (SOD1 and SOD2; Olson et al., 2017a), catalase (Olson et al., 2017b), thioredoxin reductase (TrxR) and glutaredoxin/glutathionine reductase (Grx/GSHR) systems (Doka et al. 2016). However, while these antioxidant systems irreversibly inactivate H_2O_2, they have variable effects on RSS. SOD1, SOD2 and catalase oxidize H_2S to polysulfides that are more biologically active than the original H_2S, whereas the Trx, Grx/GSHR pathways regenerate H_2S from polysulfides. This H_2S can once again be reoxidized back to polysulfides, an example of biological recycling. Furthermore, in hypoxia catalase can produce H_2S from a variety of sulfur-bearing molecules including sulfur dioxide (SO_2), thioredoxin and polysulfides in garlic. The oxygen tension (P_{O_2}) at which catalase switches from a sulfur oxidase to a sulfur reductase is around 20 mmHg, which is close to the P_{O_2} where hemoglobin unloads half its oxygen (~26 mmHg). These observations suggest that catalase-mediated sulfur metabolism is important in modern vertebrates as catalase is abundant in red blood cells and production of vasodilator H_2S will increase tissue perfusion and help match perfusion to metabolism.

5. ROS and RSS signaling

Both oxygen and sulfur signal via similar reactive species, H_2O_2 and H_2S_2, respectively, although small organic persulfides (RS_2H, where R is cysteine or glutathione) also convey RSS signals. Reactive cysteines on regulatory proteins are their primary targets (Fig. 3A-C).

ROS signaling with H_2O_2 initially forms a sulfenic acid (SOH) that will modify the function of a cysteine-based regulatory protein (Sies and Jones 2020). Additional H_2O_2 forms sulfinic (SO_2H) and sulfonic acids (SO_3H). Sulfenic acid cysteines are readily reduced back to the thiol, which restores protein function; it is not always possible to reduce sulfinic acids and sulfonic acids cannot be reduced, which targets the protein for degradation. Sulfenic cysteines may also form a disulfide bond with an adjacent thiol. Although the sulfenic sulfur can be redox recycled the signaling peroxide cannot; peroxide signaling is essentially 'one and done'.

RSS signaling has some similarities to ROS signaling but there are far more possibilities (Fig. 3C-E). The sulfur-bearing messenger may be an inorganic persulfide (RSSH, where R = H), or an organic persulfide (RSSH, where R = cysteine or glutathione). This persulfidates the cysteine sulfur on the regulatory protein, which affects protein function, essentially identical to that of a sulfeinc acid. Because the sulfur of both the protein cysteine and H_2S are in their most reduced form, the H_2S must first be oxidized to the persulfide before reacting with protein cysteines. This can occur via one-electron oxidation of two H_2S or two-electron oxidation of one H_2S (Benchoam et al. 2019). One-electron

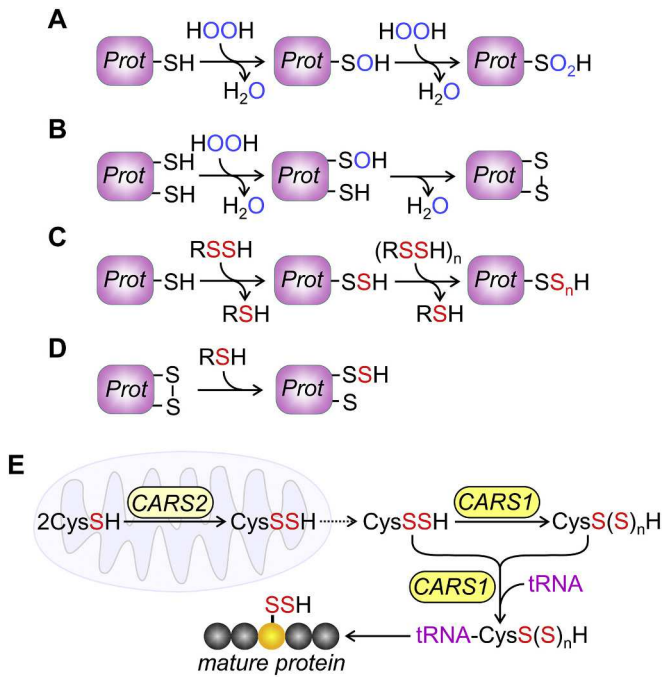

Fig. 3. ROS versus RSS signaling. Both ROS and RSS signal via redox reactions with the sulfur (S) in reactive cysteines on regulatory proteins. (A) Most ROS signaling is via H_2O_2 (HOOH) that forms a sulfenic acid (SOH), additional H_2O_2 forms sulfinic (SO_2H) and sulfonic acids (SO_3H, not shown); formation of the latter irreversibly targets the protein for degradation. (B) Sulfenic cysteines may interact with an adjacent reduced cysteine sulfur (SH) to form disulfides. RSS signals in much the same way, but there are far more possibilities. (C) Sulfur from an inorganic persulfide (RSSH, where R = H) or an organic persulfide (RSSH, where R = cysteine or glutathione) is transferred to the protein cysteine producing a protein persulfide. As many as five or six sulfur molecules may be added via this process and they may serve as a storage depot on proteins, cysteine or glutathione and reused in future sulfur transfer reactions. (D) Reduced sulfur (RSH, R = H, cysteine, glutathione) may reduce disulfide bonds. (E) RSS signaling at the level of protein translation. Cysteinyl tRNA synthase in the mitochondrion (CARS-2) catalyzes the production of cysteine persulfide (CysSS) from two mitochondrial Cys. CysSS is then exported out of the mitochondrion where cytosolic CARS (CARS-1) catalyzes the formation of tRNA-CysSS that is then directly incorporated into proteins. CARS-2 can also catalyze the formation of additional Cys polysulfides ($CysSS_n$, where n = 2–5) that can also be incorporated into proteins. CARS2 appears to account for the majority of mitochondrial and cytoplasmic polysulfides.

oxidants including radicals (superoxide, nitric oxide, hydroxyl, nitrogen dioxide, carbonate), a number of metal-centered proteins and transition metals, and to a limited extent oxygen itself, oxidize H_2S to a thiyl (sulfiyl) radical. Two thiyl radicals can then combine to form H_2S_2. In a two-electron oxidation the H_2S is first oxidized to a sulfenic acid (HSOH) that then reacts with a second H_2S to form H_2S_2 and H_2O. Biologically relevant two electron oxidants include hypochlorous acid (HOCl), a few chloramines, peroxynitrate (ONOOH) and peroxide (H_2O_2). Sulfur signaling is not limited to a 'one and done' process as the sulfur can be transferred from the protein (which restores its original function) to another thiol such as cysteine or glutathione and recycled (Kohl et al. 2019). Furthermore, as many as six additional sulfur molecules may be stored in this way, providing a large sulfur sink for immediate use. Although less well studied, H_2S may also reduce disulfide bonds.

Unlike ROS, RSS also signals at the level of protein translation (Fig. 3E). A mitochondrial cysteinyl tRNA synthase (CARS2) catalyzes the transfer of sulfur from one cysteine to another forming cysteine persulfide (CysSS) that is then exported out to the cytosol. Here a cytosolic CARS (CARS1) catalyzes the formation of tRNA-CysSS that is then directly incorporated into proteins. CARS1 can also catalyze the formation of additional Cys polysulfides ($CysSS_n$, where $n = 2–5$) that can also be incorporated into proteins (Akaike et al. 2017). This cysteine persulfide has even been proposed to be the 22nd amino acid. Although debatable, it has been estimated that as much as 70–80% of the protein Cys may be *per*-polysulfdated (Bianco et al. 2018; Fukuto et al. 2018).

6. ROS or RSS: Parallel or identical mechanisms

The similarities between ROS and RSS suggest that they may either mediate common homeostatic processes or one may be mistaken for the other. The facts that methods designed to measure ROS may detect RSS (and some with greater sensitivity), and that oxygen levels in many experiments and standard cell culture conditions are considerably greater than those found in vivo, favor an increased role for RSS (Olson 2020). This is illustrated in the following examples.

6.1. Oxygen sensing

Aerobic organisms must be able to detect oxygen and respond to oxygen availability or make appropriate metabolic adjustments, a critical attribute well recognized by August Krogh (Krogh 1919). Vertebrate blood vessels have the intrinsic ability to 'sense' oxygen. This enables systemic vessels to match perfusion with metabolism and pulmonary vessels match perfusion with ventilation; in the latter, a decrease in regional ventilation initiates hypoxic pulmonary vasoconstriction (HPV) to prevent deoxygenated blood from entering the systemic circulation (Fig. 4A-C). However, HPV resulting from global hypoxia, such as that associated with life at high altitude, pulmonary disease or hypoventilation syndromes (e.g., sleep apnea) can produce pulmonary hypertension and adversely affect function of the right heart. Vasodilators are ineffective in this instance as they will concomitantly produce systemic hypotension. Although there has been intense interest in identifying and targeting the pulmonary oxygen sensing mechanism for therapeutic purposes, this has not yet been completely successful. The leading contender in HVP involves H_2O_2 production (Fig. 4E; Sylvester et al. 2012).

We became interested in this problem when we first noticed a robust hypoxic vasoconstriction in hagfish and lamprey aortas, and using 'Kroghian' principles we developed the theory that H_2S metabolism is an effective oxygen sensor (Fig. 4F; Olson, 2015). Perhaps not surprisingly, the H_2S hypothesis is very similar to the H_2O_2 hypothesis. However, unlike H_2O_2, the H_2S mechanism can also explain why diving mammals do not experience pulmonary hypertension because both hypoxia and H_2S uniquely dilate these vessels; observations that also challenge the paradigm that HPV is a unique property of the mammalian pulmonary circulation.

6.2. Antioxidant defenses

The Kelch-like ECH-associated protein 1:Nuclear factor erythroid-derived factor 2-related factor 2 (Keap1:Nrf2) pathway is well recognized as a master regulator of antioxidant defenses (Fig. 5A; Lennicke et al. 2015). In the canonical ROS-activated hypothesis, a regulatory cysteine on Keap1 is sulfenylated by H_2O_2, which frees it from Nrf2 and allows the latter to translocate to the nucleus and initiate production of a variety of antioxidant defenses via nuclear antioxidant response elements (ARE). However, Keap1 can also be persulfidated by H_2S_2 and this has identical ARE-activating actions (Yang et al. 2013). Thus, it is difficult, if not impossible to distinguish between ROS and RSS activating factors in cells. This also raises a more fundamental question, what is the primary function of the Keap1:Nrf2 system, does it counter elevated ROS or does it regulate sulfur metabolism?

6.3. Health benefits of nutraceuticals

It is generally accepted that compounds in tea and colored berries

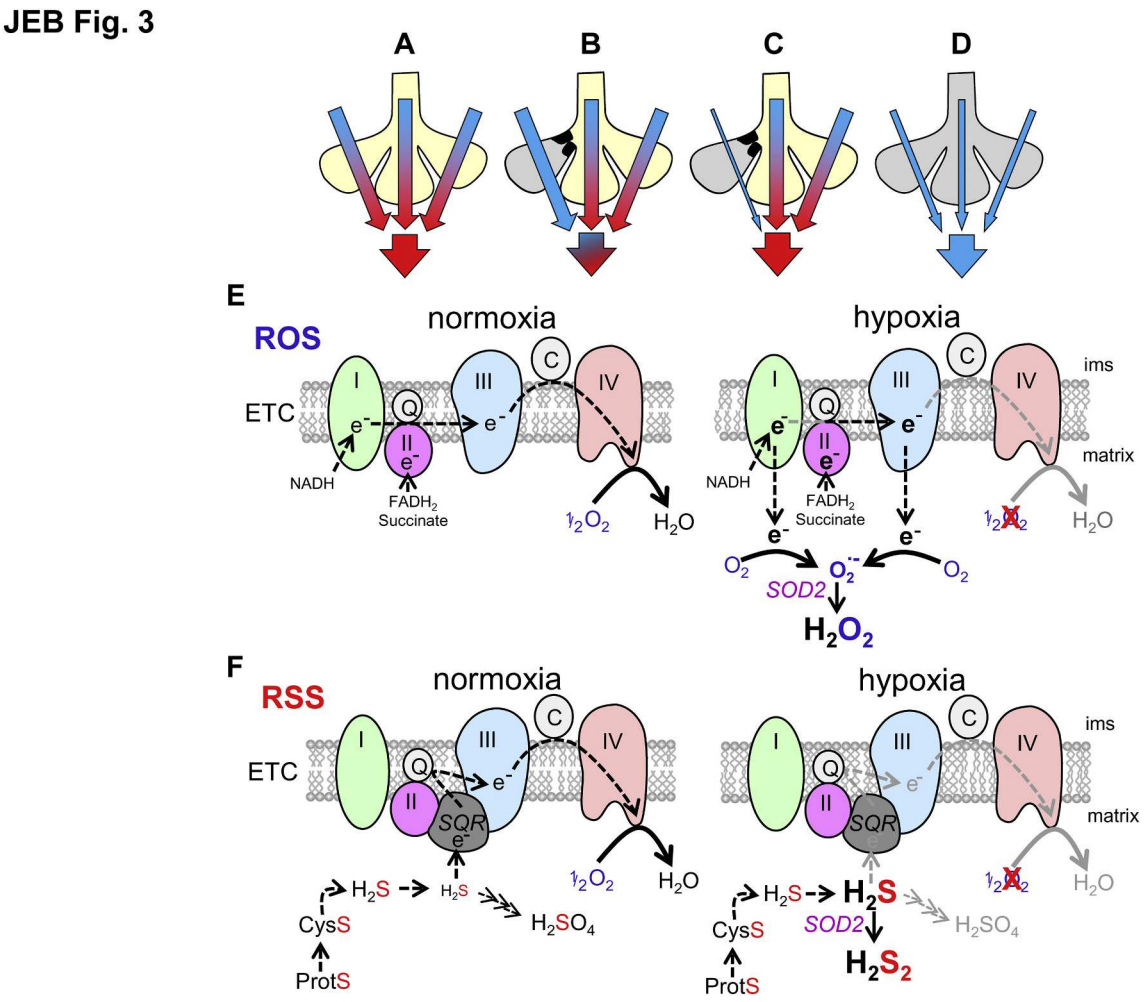

Fig. 4. Hypoxic pulmonary vasoconstriction (HPV) and proposed mechanisms for oxygen sensing. (A) Blood oxygenation is uniform (blue-red arrows) in normal, well-ventilated lungs (yellow). (B) If blood flow is maintained in an airway-obstructed region (gray) deoxygenated blood (blue arrow) mixes with oxygenated blood partially desaturating pulmonary venous blood (large arrow). (C) Hypoxic vasoconstriction (thin arrow) prevents blood flow through unventilated lung and restores pulmonary venous oxygenation. (D) Global hypoxia produces extensive hypoxic vasoconstriction which increases pulmonary vascular resistance and pulmonary arterial pressure thereby putting strain on the right ventricle. (E) ROS theory for oxygen sensing. Normal electron flow down the mitochondrial electron transport chain (ETC; left panel) is prevented in hypoxia (right panel) causing electron to back up and as they leak from complexes I and III they reduce residual oxygen to superoxide ($O_2^{\cdot-}$). Superoxide dismutase (SOD2) dismutes $O_2^{\cdot-}$ to O_2 and H_2O_2; the latter diffuses out of the mitochrondrion and initiates HPV. (F) RSS theory for oxygen sensing. In normoxia (left panel) H_2S constitutively generated from protein catabolism (ProtS) to cysteine (CysS) is oxidized by sulfide:quinone oxidoreductase (SQR) and electrons are shuttled down the ETC. Hypoxia (right panel) prevents this causing an increase in H_2S which is then oxidized by SOD1 to a persulfide (H_2S_2) that diffuses out of the mitochondrion and initiates HPV. E, F modified from Olson 2020, with permission. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

convey numerous health benefits. Many of the effects of these benefits are attributed to the antioxidant properties of polyphenolic compounds that scavenge radical species (Fig. 5B; Fan et al. 2017). However, these polyphenols, such as the green tea catechins, are also subjected to autoxidation and once oxidized they will oxidize H_2S to H_2S_2 and longer chain polysulfides (Olson et al. 2020). These polysulfides can then activate the classical antioxidant responses (cf. Fig. 5A). The relative importance of these polyphenols in ROS or RSS metabolism remains to be determined, but once resolved will lay the groundwork for 'designer nutraceuticals' that can be targeted to specific pathologies.

7. ROS vs RSS: Either, both, and under what conditions?

The obvious pressing question is the relative contribution(s) of ROS and RSS in physiology and pathophysiology. Perhaps the greatest impediment to answering this question thus far is the lack of unambiguous measurements of their intracellular concentration and distribution. This, as well as an understanding of their signaling mechanisms and metabolism has been further confounded by the nearly universal practice of performing biochemical and biological experiments in room air (21% O_2). Under these conditions dissolved oxygen concentration can be as high as 260 μM. This is, at minimum, 3 to 6 times greater than that experienced by a typical cell and 20 to 40 times that experienced by a mitochondrion. Oxygen and reduced sulfur do not coexist in either the environment or in cells which suggests that our current understanding of ROS and RSS has been experimentally biased in favor of the former at the expense of the latter. To paraphrase August Krogh 'For such a large problem there are experimental conditions with which they should be most appropriately studied'. I think more attention should be paid to this aspect of experimental design and I believe this is another advantage of Comparative Physiology.

Declaration of Competing Interest

The author has no conflicts of interest.

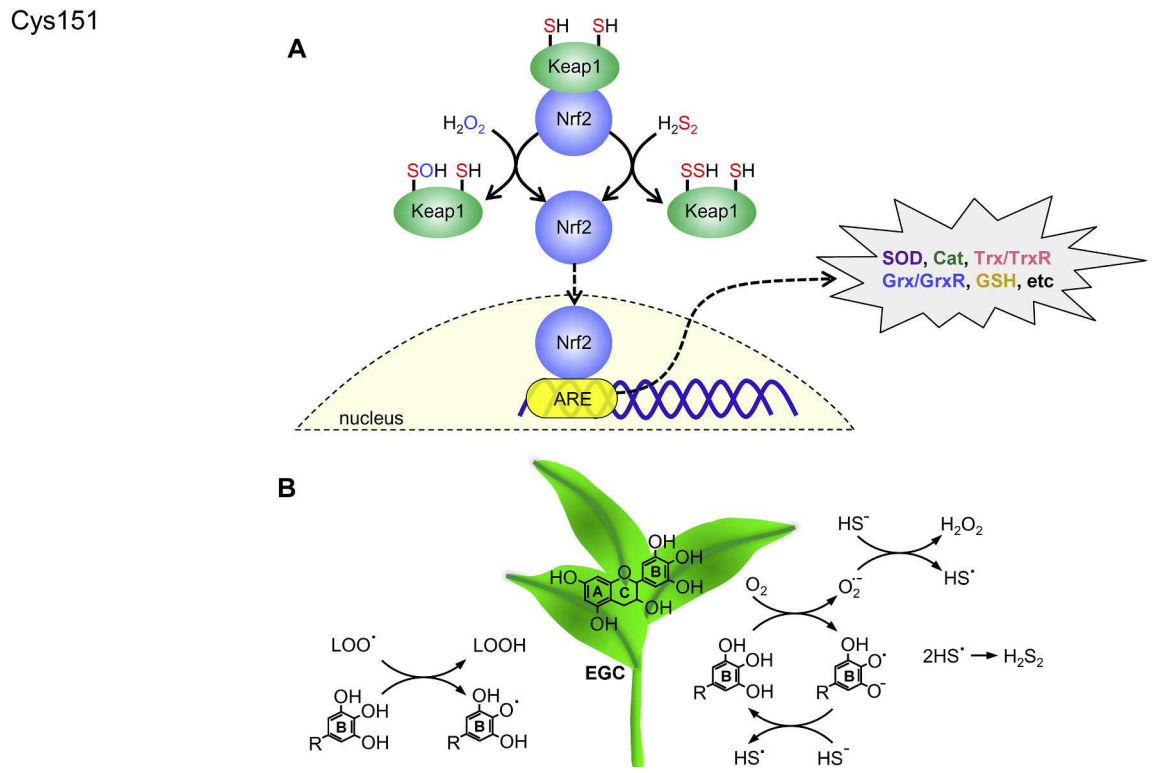

Fig. 5. Common ROS and RSS signaling mechanisms. (A) Hydrogen peroxide (H_2O_2) sulfenylates cysteine on Keap1, freeing Nrf2 which diffuses into the nucleus and initiates the antioxidant response elements (ARE) that increase production of antioxidant defenses including superoxide dismutases (SOD), catalase (Cat), thioredoxin/thioredoxin reductase (Trx/TrxR), glutaredoxin/glutaredoxin reductase (Grx/GrxR) and glutathione (GSH). Hydrogen persulfide (H_2S_2) persulfidates Keap1 cysteine producing identical responses. (B) Mechanisms of action of 'antioxidant' green tea epigallocatechin (EGC) in cells. Left: Free radical antioxidant scavenging. The EGC B ring donates an electron to a lipid peroxide radical while the relative stability of the EGC B ring semiquinone radical prevents further oxidation reactions. Right: Autoxidation of the B ring produces a semiquinone radical and superoxide, both, or either of which can react with H_2S to produce thiyl radicals. Two thiyl radicals will spontaneously combine to produce the antioxidant persulfide (H_2O_2). A, modified from Olson 2020, with permission; B, modified from Olson et al. 2020. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Acknowledgments

The author wishes to acknowledge the numerous colleagues and students that have contributed to this research. This work was supported by National Science Foundation Grant, IOS2012106.

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